

AMENDMENTS TO THE CLAIMS

1. (Currently amended) An attenuated influenza virus comprising a genomic nucleic acid segment which comprises 5' and 3' non-coding regions providing a mutated duplex region of an influenza virus RNA genomic segment operably-linked to a protein coding sequence for an influenza viral protein ~~or a functional modification of said protein~~, wherein said duplex region has one or two base-pair substitutions compared to unmutated duplex region such that expression of said protein-coding sequence in cells infected by said virus is reduced to give an attenuated phenotype.

2. (Previously presented) The virus of claim 1, wherein said virus exhibits a reduction in plaque titre compared to the parent wild-type virus on one or more type of cells selected from the group consisting of Madin-Darby bovine kidney (MDBK) cells, Madin-Darby canine Kidney (MDCK) cells and Vero cells.

3. (Previously presented) The virus of claim 2, wherein said virus exhibits at least about one log reduction in plaque titre compared to the parent wild-type virus on MDBK cells.

4. (Previously presented) The virus of claim 2, wherein said virus exhibits at least about a 3 to 4 log reduction in plaque titre compared to the parent wild-type virus on MDCK cells and Vero cells.

5. (Previously presented) The virus of claim 1, wherein said genomic nucleic acid segment is a mutated native influenza virus genomic RNA segment.

6. (Previously presented) The virus of claim 1, wherein said virus is an attenuated influenza virus of type A and said nucleic acid segment is a mutated influenza A virus genomic RNA segment having a mutation C to A at position 11 from the 3'-terminus of the native parent segment and a mutation G to U at position 12' from the 5'-terminus of the native parent segment, or functionally equivalent substitutions at the same positions, so as to provide an attenuating base-pair substitution in the non-coding duplex region.

7. (Previously presented) The virus of claim 6, wherein said nucleic acid segment further comprises a mutation U to G at position 10 from the 3' terminus of the native parent

segment and a mutation A to C at position 11' from the 5' terminus of the native parent segment, or functionally equivalent substitutions at the same positions, so as to provide an additional base-pair substitution in the non-coding duplex region.

8. (Previously presented) The virus of claim 6, wherein said nucleic acid segment encodes neuraminidase (NA) or a functional modification thereof.

9. (Previously presented) The virus of claim 1, wherein said virus is a wild-type virus which has been attenuated by said base-pair substitution(s).

10. (Previously presented) The virus of claim 1 further comprising a heterologous coding sequence capable of being expressed in target cells.

11. (Previously presented) The virus of claim 10, wherein said heterologous coding sequence encodes an antigenic peptide or polypeptide capable of stimulating an immune response to a pathogenic agent.

12. (Previously presented) The virus of claim 6 or claim 7, wherein said [wild-type] virus is an attenuated influenza A/WSN/33 and wherein said nucleic acid segment encodes neuraminidase (NA) or a functional modification thereof.

13. (Currently amended) A nucleic acid consisting of a genomic nucleic acid segment which comprises 5' and 3' non-coding regions providing a mutated duplex region of an influenza virus RNA genomic segment operably-linked to a protein coding sequence for an influenza viral protein ~~or a functional modification of said protein~~, wherein said duplex region has one or two base-pair substitutions compared to unmutated duplex region such that expression of said protein-coding sequence in cells infected by said virus is reduced to give an attenuated phenotype.

14. (Previously presented) A DNA sequence capable of transcription to provide a nucleic acid according to claim 13.

15. (Previously presented) A plasmid comprising the DNA sequence of claim 14.

16. (Previously presented) A ribonucleoprotein (RNP) complex comprising the nucleic acid of claim 13 complexed with polymerase proteins and nucleoprotein of an influenza virus.

17. (Currently amended) An ex vivo cell infected by an attenuated influenza virus comprising a genomic nucleic acid segment which comprises 5' and 3' non-coding regions providing a mutated duplex region of an influenza virus RNA genomic segment operably-linked to a protein coding sequence for an influenza viral protein ~~or a functional modification of said protein~~, wherein said duplex region has one or two base-pair substitutions compared to unmutated duplex region such that expression of said protein-coding sequence in cells infected by said virus is reduced to give an attenuated phenotype.

18. (Currently amended) A vaccine comprising an attenuated influenza virus comprising a genomic nucleic acid segment which comprises 5' and 3' non-coding regions providing a mutated duplex region of an influenza viral protein ~~or a functional modification of said protein~~, wherein said duplex region has one or two base-pair substitutions compared to unmutated duplex region such that expression of said protein-coding sequence in cells infected by said virus is reduced to give an attenuated phenotype.

19. (Previously presented) The vaccine of claim 18 wherein said attenuated influenza virus additionally comprises a heterologous coding sequence which encodes an antigenic peptide or polypeptide capable of stimulating an immune response to a pathogenic agent.

20. (Previously presented) A pharmaceutical composition comprising the virus of claim 10 in combination with a pharmaceutically acceptable carrier or diluent for delivery of said heterologous coding sequence to target cells.

21. (Previously presented) A pharmaceutical composition comprising cells infected with the virus of claim 10 in combination with a pharmaceutically acceptable carrier or diluent.

22. (Currently amended) A method of preparing an attenuated influenza virus comprising the steps of:

constructing a genomic nucleic acid segment which comprises 5' and 3' non-coding regions providing a mutated duplex region of an influenza virus RNA genomic segment operably-linked to a protein coding sequence for an influenza viral protein ~~or a functional modification of said protein~~, wherein said duplex region has one or two base-pair substitutions compared to unmutated duplex region such that expression of said protein-coding sequence in cells infected by said virus is reduced to give an attenuated phenotype;

providing to a host cell said genomic nucleic acid segment together with the additional genomic nucleic acid segments for said virus under conditions whereby said segments are packaged into a viral particle; and

selecting said virus.

23. (Currently amended) A method of preparing an influenza virus by a helper virus based influenza gene rescue system comprising the steps of:

transfecting a host cell with a helper virus, wherein said helper virus is an attenuated influenza virus comprising a genomic nucleic acid segment which comprises 5' and 3' non-coding regions providing a mutated duplex region of an influenza virus RNA genomic segment operably-linked to a protein coding sequence for an influenza viral protein ~~or a functional modification of said protein~~, wherein said duplex region has one or two base-pair substitutions compared to unmutated duplex region such that expression of said protein-coding sequence in cells infected by said virus is reduced to give an attenuated phenotype;

transfecting said host cell with a nucleic acid segment of an influenza virus to be rescued, wherein said nucleic acid segment also encodes said protein or a functional modification thereof; and

selecting viruses containing said nucleic acid segment on the basis of increased growth compared with the helper virus on cells of a selected type.

24. (Previously presented) The method of claim 23, wherein said helper virus is an attenuated influenza A virus and said genomic nucleic acid segment and said nucleic acid segment encode neuraminidase or a functional modification thereof.

25. (Previously presented) The method of claim 24, wherein said helper virus is attenuated influenza A/WSN/33 having in its NA-encoding genomic nucleic acid segment a mutation C to A at position 11 from the 3'-terminus of the native parent segment, a mutation G to U at position 12' from the 5'-terminus of the native parent segment, a mutation U to G at position 10 from the 3' terminus of the native parent segment and a mutation A to C at position 11' from the 5' terminus of the native parent segment, and wherein said host cell is a Vero cell.

26. (Withdrawn) A method of stimulating an immune response against an influenza virus, comprising the step of administering to an animal in an immunizing mode the attenuated influenza virus of claim 1.

27. (Withdrawn) A method of delivering a heterologous coding sequence to cells comprising infecting said cells with the virus of claim 10.

28. (Previously presented) The method of claim 23, wherein said nucleic acid segment of an influenza virus is complexed with polymerase proteins and nucleoproteins to form a RNA complex which is transfected.

29. (Previously presented) The nucleic acid of claim 13, wherein said genomic nucleic acid segment is a mutated native influenza virus genomic RNA segment.

30. (Previously presented) The nucleic acid of claim 13, wherein said nucleic acid segment is a mutated influenza A virus genomic RNA segment having a mutation C to A at position 11 from the 3'-terminus of the native parent segment and a mutation G to U at position 12' from the 5'-terminus of the native parent segment, or functionally equivalent substitutions at the same positions, so as to provide an attenuating base-pair substitution in the non-coding duplex region.

31. (Previously presented) The nucleic acid of claim 30, wherein said nucleic acid segment further comprises a mutation U to G at position 10 from the 3' terminus of the native parent segment and a mutation A to C at position 11' from the 5' terminus of the native parent segment, or functionally equivalent substitutions at the same positions, so as to provide an additional base-pair substitution in the non-coding duplex region.

32. (Previously presented) The nucleic acid of claim 30, wherein said nucleic acid segment encodes neuraminidase (NA) or a functional modification thereof.

33. (Previously presented) The ex vivo cell of claim 17, wherein said genomic nucleic acid segment is a mutated native influenza virus genomic RNA segment.

34. (Previously presented) The ex vivo cell of claim 17, wherein said virus is an attenuated influenza virus of type A and said nucleic acid segment is a mutated influenza A virus genomic RNA segment having a mutation C to A at position 11 from 3'-terminus of the native parent segment and a mutation G to U at position 12' from the 5'-terminus of the native parent segment, or functionally equivalent substitutions at the same positions, so as to provide an attenuating base-pair substitution in the non-coding duplex region.

35. (Previously presented) The ex vivo cell of claim 34, wherein said nucleic acid segment further comprises a mutation U to G at position 10 from the 3' terminus of the native parent segment and a mutation A to C at position 11' from the 5' terminus of the native parent segment, or functionally equivalent substitutions at the same positions, so as to provide an additional base-pair substitution in the non-coding duplex region.

36. (Previously presented) The ex vivo cell of claim 34, wherein said nucleic acid segment encodes neuraminidase (NA) or a functional modification thereof.

37. (Previously presented) The ex vivo cell of claim 17, wherein said virus is a wild-type virus which has been attenuated by said base-pair substitution.

38. (Previously presented) The ex vivo cell of claim 17 wherein said virus further comprises a heterologous coding sequence capable of being expressed in target cells.

39. (Previously presented) The ex vivo cell of claim 38, wherein said heterologous coding sequence encodes an antigenic peptide or polypeptide capable of stimulating an immune response to a pathogenic agent.

40. (Previously presented) The ex vivo cell of claim 34 or claim 35, wherein said virus is an attenuated influenza A/WSN/33 and said nucleic acid segment encodes neuraminidase (NA) or a functional modification thereof.

Claims 41-47 (Canceled)

48. (Previously presented) The virus of claim 6 wherein said genomic nucleic acid segment is a mutated NS1-encoding genomic nucleic acid segment.

49. (Previously presented) The virus of claim 6 wherein said genomic nucleic acid segment is a mutated PA-encoding genomic nucleic acid segment.

50. (Previously presented) The virus of claim 6 wherein said genomic nucleic acid segment is a mutated PB2-encoding genomic nucleic acid segment.

51. (Previously presented) The nucleic acid of claim 30 which is a mutated NS1-encoding genomic nucleic acid segment.

52. (Previously presented) The nucleic acid of claim 30 which is a mutated PA-encoding genomic nucleic acid segment.

53. (Previously presented) The nucleic acid segment of claim 30 which is a mutated PB2-encoding genomic nucleic acid segment.

54. (Previously presented) The ex vivo cell of claim 34 wherein said nucleic acid segment is a mutated NS1-encoding nucleic acid segment.

55. (Previously presented) The ex vivo cell of claim 34 wherein said nucleic acid segment is a mutated PA-encoding genomic nucleic acid segment.

56. (Previously presented) The ex vivo cell of claim 34 wherein said nucleic acid segment is a mutated PB2-encoding nucleic acid segment.

57. (New) An attenuated influenza virus comprising a genomic nucleic acid segment which comprises 5' and 3' non coding regions providing a mutated duplex region of an influenza virus RNA genomic segment operably linked to a protein coding sequence for an influenza viral protein, wherein said duplex region has at least one base pair substitution at position 11 from the 3' terminus and 12' from the 5' terminus of the native segment, or at position 10 from the 3' terminus and position 11' from the 5' terminus of the native segment.

58. (New) The attenuated virus of claim 57, wherein the base pair substitution is at position 10 from the 3' terminus and position 11' from the 5' terminus of the native segment.

59. (New) The attenuated virus of claim 57, wherein the base pair substitution is at position 11 from the 3' terminus and 12' from the 5' terminus of the native segment.

60. (New) The attenuated virus of claim 57 further comprising a base pair substitution at position 12 from the 3' terminus and position 13 from the 5' terminus of the native segment.